extra-column volume compromises separation efficiency. Polymer-based, or PEEK®, tubing and fittings throughout the chromatographic flow path are becoming very popular. PEEK tubing is available in a wide range of sizes, is colour-coded for ease of size selection, is easy to work with, is tolerant to a wide range of buffer and solvent conditions, and is impermeable to oxygen.

The effects of band-broadening are not important prior to injection; therefore, the inner diameter of the tubing from the pump to the injector/autosampler should be as large (i.e. typically 0.030 inch i.d.) as possible (i.e. to reduce system back-pressure) while maintaining high-pressure strength. From the injector to the column to the detector, all tubing should be as short as possible and of the narrowest diameter available to minimize extra-column effects. Post-injector tubing diameters are often 0.01–0.005 inch i.d.

Related HPLC Techniques

The HPLC system described above was based on the dimensions of a typical analytical system, otherwise known as 'normal-bore chromatography'. The same basic components and designs are used in both micro- (i.e. column dimensions of 1–2 mm i.d.) and preparative systems, which have much larger column dimensions.

Microchromatographic techniques include microbore, nanobore, and capillary chromatography, which have column inner diameters of 2 to 1 mm, 1 to 0.3 mm, and less than 300 μm, respectively. Most importantly, microchromatographic systems require smaller extra-column volumes than normal-bore systems. Hence, great pains should be taken to use the smallest inner-diameter tubing available, zero dead-volume fittings, smaller injection volumes, and smaller detection cell volumes. Microchromatographic systems are employed when sample volume is limited.

If compound purification or isolation is intended, preparative chromatography is used. Since a large quantity of material is to be injected in order to isolate a significant quantity of analyte, column capacity and, hence, column diameters are larger. Column diameters in preparative HPLC can be on the order of metres. The high flow rates needed for these large-scale systems require the use of special pumps and larger tubing throughout.

See also: **II/Chromatography: Liquid:** Derivatization; Detectors: Ultraviolet and Visible Detection; Mechanisms: Ion Chromatography.

Further Reading

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Ion Chromatography: Mechanisms

See **II / CHROMATOGRAPHY: LIQUID / Mechanisms: Ion Chromatography**

Ion Pair Liquid Chromatography

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Ion pair chromatography is a widely used reversed phase liquid chromatographic technique for the separation of ionic analytes. Retention of ions in ion pair chromatography is determined by many experimental parameters and it therefore provides an effective tool for obtaining chromatographic separation between ionic analytes. A major part of this article discusses the influence of the most important

parameters on retention. A short presentation of the areas of application of ion pair chromatography, as well as some practical guidelines for its use, is also included.

Introduction to Ion Pair Chromatography

Ion pair chromatography (IPC) is an effective reversed-phase liquid chromatographic (RPLC) technique for separation of organic ions and partly ionized organic analytes. The technique utilizes the same types of stationary phases and mobile phases as RPLC; the main characteristic for IPC is that an ion pair reagent is added to the mobile phase. The ion pair reagent is usually an alkylsulfonate, an alkylsulfate or an alkylammonium salt. The high efficiency of RPC columns compared with columns used in ion exchange or ion chromatography also makes IPC a valuable alternative to these techniques.

The purpose of adding an ion pair reagent to the mobile phase is usually to change the retention time of ionic analytes. By varying the mobile phase concentration of the ion pair reagent, the retention factor for an oppositely mono-charged analyte can be continuously increased by a factor of 10–20 compared to the value with no added ion pair reagent. Correspondingly, it is possible to continuously reduce the retention factor for a similarly mono-charged analyte by a factor of 10–20. The retention factor for non-charged analytes is usually more or less unaffected by the presence of the ion pair reagent.

Ion pair extraction, i.e. extraction of ionized solutes into organic phases by adding an oppositely charged ion to the system, has been used for many decades. In this technique, the distribution of an ion into the organic phase is enhanced by the formation of an ion pair between the two oppositely charged ions. The pioneering work in IPC by Schill and coworkers in Uppsala was performed in the liquid–liquid partition mode. The extraction of an ion pair to the organic phase was considered to be the cause of retention, and the name ion pair chromatography originates from this early application. When covalently bonded non-polar stationary phases were introduced, important contributions to the further development of IPC were, among others, made by the research groups of Horváth, Knox, Schill and Haney.

IPC has been applied in almost all areas of analytical chemistry where chromatography is used. Since many drugs are basic or acidic, the driving force for the development of IPC came from the pharmaceutical industry where today it is used on a routine basis. Of particular current interest is chiral separations of pharmaceutical compounds by using a chiral ion pair reagent. In IPC, water-rich mobile phases can be employed with a variety of buffers and ionic and non-ionic additives and the technique is therefore suitable for separation of important classes of biomolecules and specifically amino acids, peptides, proteins and nucleic acids. In the food industry, IPC is used for the analysis of water-soluble vitamins, caffeine, theobromine, amines, etc.

For the separation of inorganic cations and anions IPC is often used as an alternative to ion (exchange) chromatography and in these applications it is usually referred to as ion interaction chromatography. In ion interaction chromatography, the ion pair reagent is usually called the ion interaction reagent. The technique has been used, for example, in the area of environmental analysis for the separation and analysis of nitrate and nitrite. Another technique that is closely related to ion pair chromatography is micellar chromatography. Here the concentration of ion pair reagent in the mobile phase is so high that micelles are formed, i.e. the concentration of the ion pair reagent in the mobile phase is above the critical micelle concentration (CMC). Micellar chromatography is treated in a separate article.

When the analyte ion lacks properties that make it easily detectable by commonly used detectors, IPC in the indirect detection mode can be used. The basis of this method is that a constant concentration of a detectable ion pair reagent is added to the mobile phase. In the chromatographic zone, where the non-detectable analyte ion is present, a change from the otherwise constant concentration of the detectable ion pair reagent is induced by the analyte. Depending on the relative properties of the analyte and the ion pair reagent, the concentration of ion pair reagent in the analyte zone may be either higher or lower than in the mobile phase.

Retention and selectivity in reversed-phase IPC are influenced by a large number of experimental parameters. These parameters are given in **Table 1** together with a short description of their effect on retention. Briefly, for the separation of a particular set of analyte ions the parameters that are most important are type and concentration of ion pair reagent, type and concentration of organic modifier, and mobile phase pH. By varying these parameters while keeping the others constant within sensible ranges, an acceptable separation is usually obtained. A rational use of IPC is facilitated by a basic knowledge of the impact of the different parameters on retention. A more detailed analysis of the role of the most important parameters is presented below.

Table 1 Effect on retention of increasing the value of different chromatographic variables in reversed-phase ion pair chromatographic systems

Variable	*Effect on k for oppositely charged solute*	*Effect on k for similarly charged solute*
Increasing charge of analyte ion ($z_B = \pm 1,2,\ldots$)	Increases the slope of the ln k_{cB} vs ln c_A relation	Increases the absolute value of the slope of the ln k_{cB} vs ln c_A relation
Increasing charge of pairing ion ($z_A = \pm 1,2,\ldots$)	Slightly decreases the slope of the ln k_{cB} vs ln c_A relation	Slightly decreases the absolute value of the slope of the ln k_{cB} vs ln c_A relation
Increasing concentration of pairing ion (c_A)	Increases	Decreases
Increasing hydrophobicity of pairing ion (K_A)	Increases	Decreases
Increasing concentration of organic modifier (φ)	Decreases. The slope of the ln k_{cB} vs φ relationship becomes steeper compared with the regular *RP* slope	Decreases. The slope of the ln k_{cB} vs φ relationship becomes less steep compared with the regular *RP* slope
Increasing polarity of the organic modifier	The slope of the ln k_{cB} vs φ relationship decreases	The absolute value of the slope of the ln k_{cB} vs φ relationship increases
Increasing ionic strength (I)	Decreases	Increases
Type of stationary phase	A parallel shift of the ln k_{cB} vs ln c_A relationship occurs for both positively and negatively charged analytes when using different columns	
Eluent pH	Retention of the ionized form increases; it can become even larger than the regular *RP* retention of the non-ionized form	Retention of the ionized form decreases

Retention Theories for Ion Pair Chromatography

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Stoichiometric Theories

In this article IPC means liquid chromatography performed on columns with covalently bonded non-polar stationary phases, in combination with polar mobile phases containing an ion pair reagent. The earliest retention theories for IPC were stoichiometric, i.e. the retention is assumed to be caused by the formation of a 1 : 1 stoichiometric complex between the ion pair reagent and the oppositely charged analyte ion. Depending on the particular theory, complex formation is considered to take place in the mobile phase or in the bonded phase. Other theories consider the ion pair reagent to be adsorbed to the stationary phase surface and describe retention as a result of a stoichiometric ion exchange process between the analyte ion and the counter ions to the ion pair reagent. In 1981 it was pointed out by Knox and Hartwick that many of the proposed stoichiometric retention theories are thermodynamically equivalent and that it is therefore not possible to distinguish them from each other by retention measurements alone.

The physicochemical cause of retention in ion pair chromatography has been a controversial issue. Because of this disagreement many alternative names have been proposed for this technique. These include: dynamic ion exchange chromatography, ion-interaction chromatography, hetaeric chromatography, and soap chromatography. Correspondingly, many names have been used for the ion pair reagent.

Non-stoichiometric Theories

Modern retention theories recognize that the layer of the bonded phase is so thin that the surface area to bonded phase volume ratio is extremely high. As a consequence, the surface science view on adsorption of both the analyte and the ion pair reagent to the stationary phase can be applied. In these theories, the electrostatic interaction between the ion pair reagent and the analyte ion leads to a non-stoichiometric retention theory. The non-stoichiometry is a consequence of the fact that the electrostatic interaction is long ranged and therefore gives rise to a multibody interaction (**Figure 1**).

The common basis of modern retention theories is that a charged surface is created when the ion pair reagent adsorbs at the interface between the polar mobile phase and the hydrophobic stationary phase. The inorganic counterions to the ion pair reagent are territorially bound to the charged surface in a diffuse layer and a diffuse double layer is formed. Physically this results in a difference in electrostatic potential, $\Delta\Psi_0$, between the bulk of the mobile phase and the surface ($\Delta\Psi_0$ has the same physical origin as the more

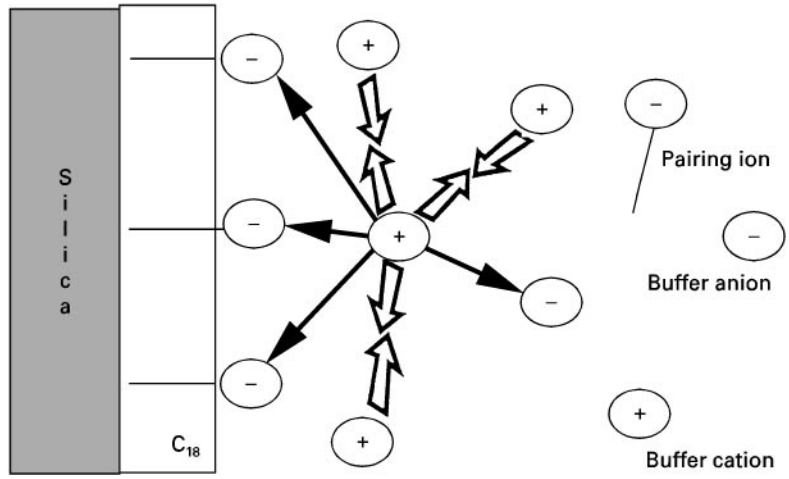

Figure 1 Schematic illustration of the long-range nature of electrostatic forces between ions in typical ion pair chromatographic systems. Open and full arrows represent electrostatic repulsion and attraction forces, respectively. (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

frequently encountered zeta-potential, although they may differ in their numerical value). For a positively charged ion pair reagent the numerical value for $\Delta\Psi_0$ is positive and for a negative reagent it is negative. Qualitatively, the charging of the stationary phase surface infers that the retention of analyte ions of opposite charge to the pairing ion increases due to electrostatic attraction. It also infers that the retention of analyte ions of similar charge to the pairing ion decreases due to electrostatic repulsion (**Figure 2**).

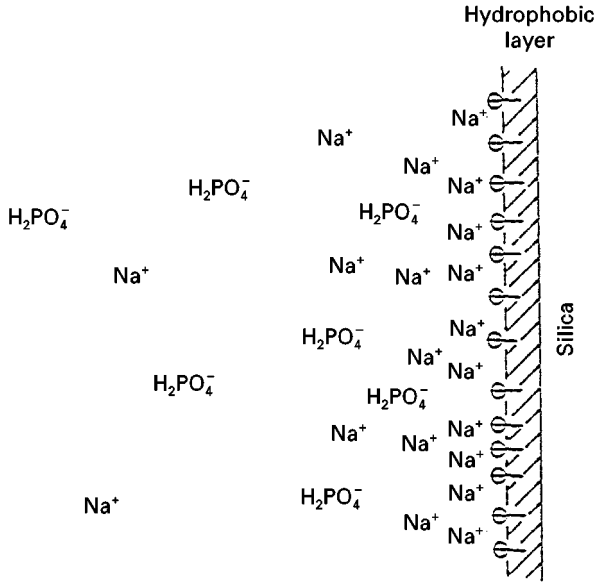

Figure 2 A schematic picture of the electrical double layer in reversed-phase ion pair chromatography. (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

Several non-stoichiometric theories have been proposed, which all use the difference in electrostatic potential between the mobile and stationary phase as a parameter that influences the retention of an ionic analyte. However, there are important differences between the theories in the physical description and also in the role that the electrostatic surface potential plays in retention. For example, in the theory proposed by Cantwell and co-workers, retention is considered to be due to a mixed ion exchange and electrostatic effect. The disagreement today among the proponents of non-stoichiometric theories seems to be on how retention is best described under conditions of high surface loadings of the ion pair reagent, i.e. when the electrostatic surface potential is higher than $\pm$ 60 mV. The disagreement is a consequence of the fact that under these conditions a number of different, not fully understood, physical phenomena may occur in the diffuse double layer.

Parameters Controlling the Retention factor in Ion Pair Chromatography

Theoretical Aspects

A simplified version of the surface adsorption double layer model for IPC provides a convenient framework for treating the influence of the various experimental parameters on the retention of medium-sized (mw = 100–300) mono- and di-charged organic ions. This is a non-stoichiometric theory developed for electrostatic surface potentials in the range 15–40 mV.

In this theory, the retention factor of an analyte ion in the absence of the ion pair reagent, k_{0B}, is related to its standard free energy of adsorption, ΔG_B^0, according to:

$$k_{0B} = \phi \cdot \exp\left(-\frac{\Delta G_B^0}{RT}\right) \quad [1]$$

where the subscript zero denotes that the concentration of the ion pair concentration reagent is zero and ϕ is the column phase ratio. To a first approximation, the presence of the ion pair reagent on the surface does not change any property of the stationary phase other than its electrostatic potential. This infers that in the presence of the ion pair reagent the standard free energy of adsorption of the analyte changes to ΔG_t^0 where:

$$\Delta G_t^0 = \Delta G_B^0 + z_B F \Delta\Psi_0 \quad [2]$$

Here z_B is the charge of the analyte ion and F is the Faraday constant. ΔG_B^0 is usually referred to as the chemical part of the free energy of adsorption and $z_B F \Delta\Psi_0$ as the electrostatic part. Assuming that the retention of the analyte is due to adsorption to the stationary phase surface only, its retention factor, k_{cB}, in the presence of the ion pair reagent becomes:

$$k_{cB} = \phi \cdot \exp\left(-\frac{\Delta G_B^0 + z_B F \Delta\Psi_0}{RT}\right) = k_{0B} \cdot \exp\left(-\frac{z_B F \Delta\Psi_0}{RT}\right) \quad [3]$$

According to equation [3], the retention of an ionic analyte in IPC is determined by its retention in the absence of the ion pair reagent, the charge of the analyte ion and the electrostatic surface potential induced by the ion pair reagent. The influence of different experimental variables on the retention can for most cases be rationalized and quantitatively or semi-quantitatively described from eqn [3]. Deviations may occur, for example, when the value for k_{0B} is less than ≈ 0.5, or when the surface concentration of the ion pair reagent is higher than 40–50% of the monolayer capacity of the surface.

The value for $\Delta\Psi_0$ is primarily a function of the surface concentration of the ion pair reagent and of the ionic strength of the mobile phase. The relationship between these variables can be obtained from solving the Poisson–Boltzmann equation for charged surfaces in contact with an electrolyte solution. When the electrostatic surface potential is below ± 25 mV the solution shows that there is a linear relation between the surface concentration of charges and $\Delta\Psi_0$:

$$\Delta\Psi_0 = \frac{z_A n_A F}{\kappa \varepsilon_0 \varepsilon_r} \quad [4]$$

where n_A is the surface concentration of the ion pair reagent ($\mathrm{mol\,m^{-2}}$), κ is the inverse Debye length (1/m), ε_0 is the permittivity of vacuum and ε_r is the dielectric constant of the mobile phase (dimensionless). The numerical value for κ is proportional to the square root of the ionic strength of the mobile phase.

In practice, it is not the surface concentration of the ion pair reagent but its mobile phase concentration that is experimentally controlled. The relationship between these two is determined by the adsorption isotherm of the ion pair reagent onto the stationary phase surface. In analogy with the discussion concerning the adsorption of the analyte ion, the free energy of adsorption of the ion pair reagent is divided into a chemical and an electrostatic part. Physically this means that the electrostatic surface potential created by the ion pair reagent must be included in its own adsorption isotherm. The simplest is the surface potential-modified linear adsorption isotherm:

$$n_A = n_0 K_A c_A \cdot \exp\left(-\frac{z_B F \Delta\Psi_0}{RT}\right) \quad [5]$$

where n_0 is the monolayer capacity of the surface, c_A is the mobile phase concentration of the ion pair reagent and K_A is its association constant to the stationary phase. The exponential term in the equation accounts for the electrostatic repulsion induced by the adsorbed ion pair reagent itself and it gives rise to a nonlinear adsorption isotherm. Equation [5] has been experimentally verified in many different reversed phase ion pair systems and can be used when n_A is less than $0.3 n_0$.

After making some approximations it is possible to derive eqn [6] from eqns [3]–[5]

$$\ln k_{cB} = \ln k_{0B} + \frac{-z_A z_B}{(z_A^2 + 1)}\left[\ln n_0 K_A + \ln c_A - \frac{1}{2}\ln I + \text{const.}\right] \quad [6]$$

where I is the mobile phase ionic strength. This equation in a convenient way separates the influence of the different experimental variables on retention. For oppositely mono-charged analyte and ion pair reagent, the equation is approximately valid in the interval 2–10 for the k_{cB}/k_{0B} ratio.

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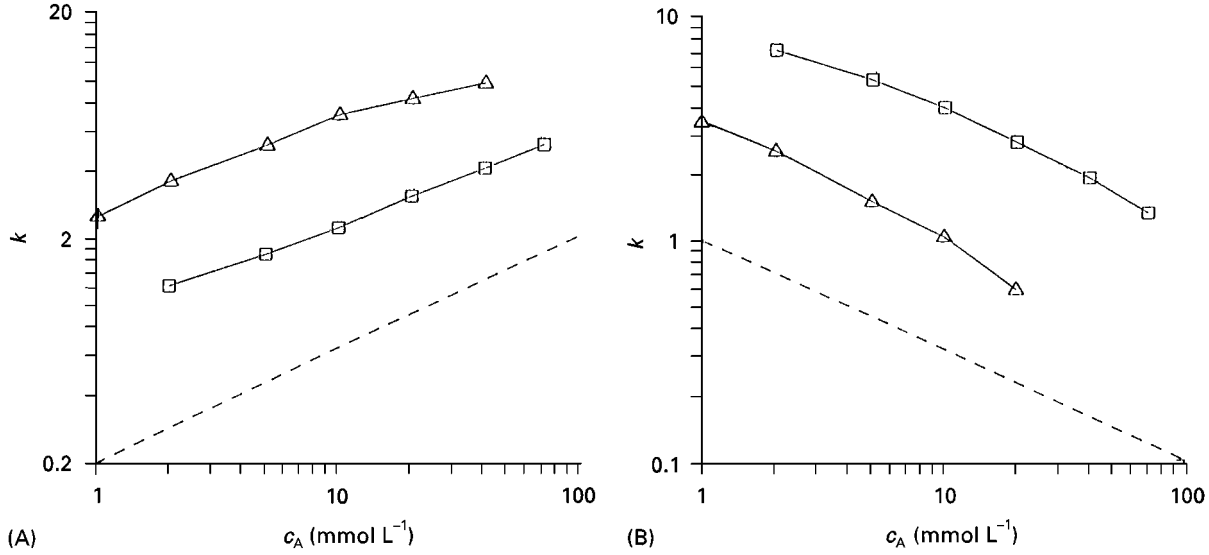

Figure 3 Retention factor for (A) dopamine and (B) naphthalenesulfonic acid as a function of the mobile phase concentration of sodium hexylsulfonate (□) and octylsulfonate (△) pairing ions. – – –, Theoretical slope. Measurements were made in methanol–aqueous phosphate buffer (pH 2.1, 10 : 90 v/v). (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

Charge of the Analyte and Ion Pair Reagent Ion

The influence of the charge of the analyte ion on retention when the mobile phase concentration of the ion pair reagent varies can be understood from eqn [6]. When $z_A = +1$ and $z_B = -1$, the equation predicts that in a ln k_{cB} vs ln c_A plot the slope of the straight line is equal to 1/2 and when $z_B = z_A = -1$ it is equal to $-1/2$. Examples of experimental data for these two cases are shown in **Figure 3**.

For multiple charged ion pair reagents, the non-linearity of the adsorption isotherm becomes more pronounced than for monocharged reagents. Quantitatively, this is described in eqns [4]–[6] through the value for z_A; when $z_A = +2$ eqn [6] predicts that the slope of the ln k_{cB} vs ln c_A plot is 2/5 for a negatively mono-charged analyte ion and 4/5 for a di-charged ion. An example is shown in **Figure 4** where the di-charged hexamethonium bromide ion is used as ion pair reagent. The solid lines in the figure corresponds to the theoretically predicted slope.

Type of Analyte Ion and Ion Pair Reagent

In a reversed phase chromatographic system, the ionized form of a molecule has a much lower retention factor than the corresponding non-ionized form. The main reason is that charged species have a lower energy in a medium of high dielectric constant (i.e. the mobile phase) than in a medium of low dielectric constant (i.e. the hydrophobic surface layer). Within a series of analyte ions of identical charge but different hydrophobicity, the retention order follows the same rules as for non-ionized analytes, i.e. the more hydrophobic analyte is more retained. This property is reflected in eqns [3] and [6] by different k_{0B} values for different fully ionized analytes.

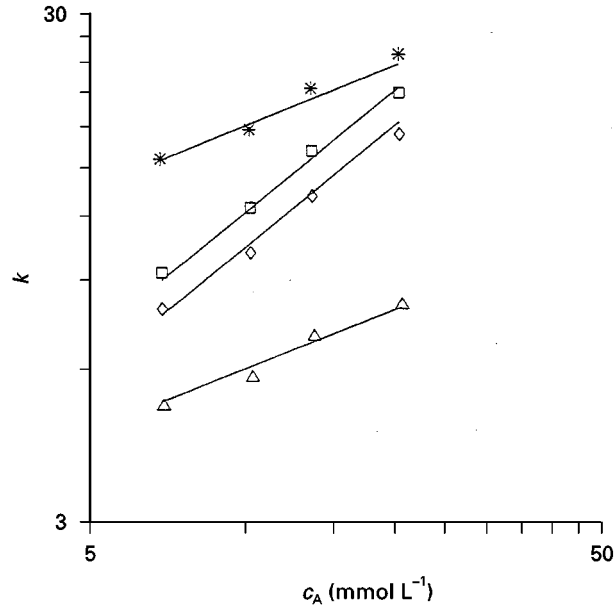

Figure 4 Retention factor data for mono-charged (△, 1-naphthylamine-4-sulfonic acid; *, 6-naphthol-2-sulfonic acid) and double-charged sulfonic acids (□, 2-naphthol-6,8-disulfonic acid; ◇, naphthalene-2,7-disulfonic acid) as a function of eluent concentration (c_A) of the double-charged pairing ion, hexamethonium bromide ($z_A = +2$). (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

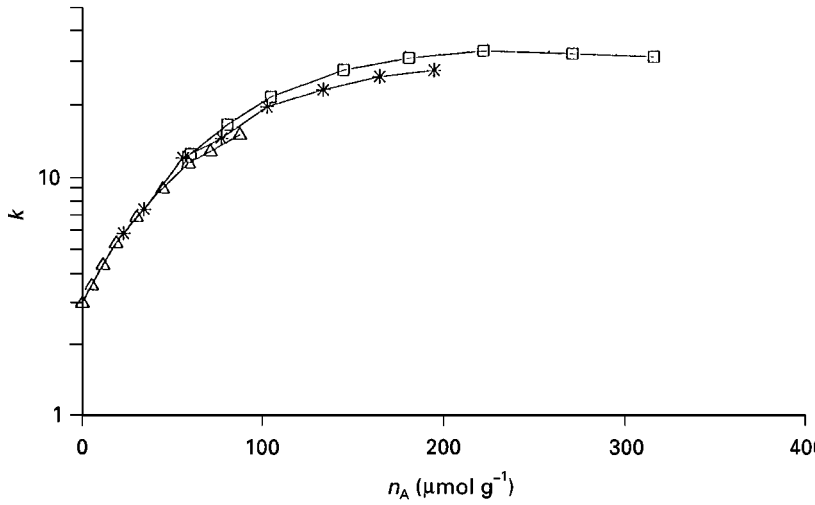

Figure 5 Retention factor of adrenaline as a function of the stationary phase surface concentration (n_A) of sodium butyl- (△), hexyl- (∗) and octyl- (□) sulfonate pairing ions measured at constant ionic strength (175 mM Na^+) of the phosphate buffer (pH 2.1) on an ODS-Hypersil column. (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

Under the condition of identical mobile phase concentration of two ion pair reagent ions of different hydrophobicity, the more hydrophobic reagent ion gives a higher retention for an oppositely charged analyte. This is attributed to higher adsorption to the stationary phase surface of the more hydrophobic reagent and a correspondingly higher induced electrostatic surface potential. Since a relative change in retention factor is primarily caused by a change in electrostatic surface potential, it is the surface concentration of the ion pair reagent, and not its detailed molecular structure, which is of importance for retention changes. This important fact is illustrated in **Figure 5** where the retention factor of adrenaline is solely a function of the surface concentration of the three different alkylsulfonates and is independent of the type of ion pair reagent.

Concentration of Organic Modifier in the Mobile Phase

In reversed-phase liquid chromatography, the effect of organic modifier concentration on the retention factor for non-charged analytes is usually described according to eqn [7]

$$\ln k_B(\varphi) = \ln k_{wB} - S_B\phi \qquad [7]$$

where k_{wB} is the retention factor for analyte B in a water–buffer mobile phase, S_B is a constant for a given analyte–solvent combination, and ϕ is the organic modifier concentration in the mobile phase. The thermodynamic interpretation of eqn [7] is that the free energy of adsorption of the analyte B to the reversed phase surface is linearly dependent on the organic modifier concentration. In an ion pair system the retention depends on the adsorption of two components, the ion pair reagent and the analyte, respectively. There are therefore two different adsorption energies, and correspondingly, two equilibrium constants for adsorption involved. The logarithm of both these equilibrium constants approximately obey equation [7]. That is, besides the effect on the analyte ion a linear relation with coefficient, S_A, describes the change in $\ln K_A$ with changing organic modifier concentration. The final result is that $\ln k$ for an analyte ion depends on both the value of S_B and S_A. For a mono-charged analyte and ion pair reagent of opposite charge, eqn [6] gives that the slope of the $\ln k_{cB}$ vs ϕ relation becomes $S_B + \frac{1}{2}S_A$, while if they are of similar charge the slope becomes $S_B - \frac{1}{2}S_A$.

In **Figure 6** the logarithm of the retention factor for a positively charged (phenylalanine) and a negatively charged (naphthalene-2-sulfonic acid) analyte is plotted as a function of the volume concentration of methanol in the absence and presence of sodium octylsulfonate, respectively. The dashed line in both figures corresponds to the slope S_B for the corresponding analyte ion, whereas in Figure 6A the solid line corresponds to the slope $S_B + \frac{1}{2}S_A$ and in Figure 6B it corresponds to the slope $S_B - \frac{1}{2}S_A$.

Effect of pH of the Mobile Phase

For weak acid and basic analytes the mobile phase pH value influences the degree of ionization and con-

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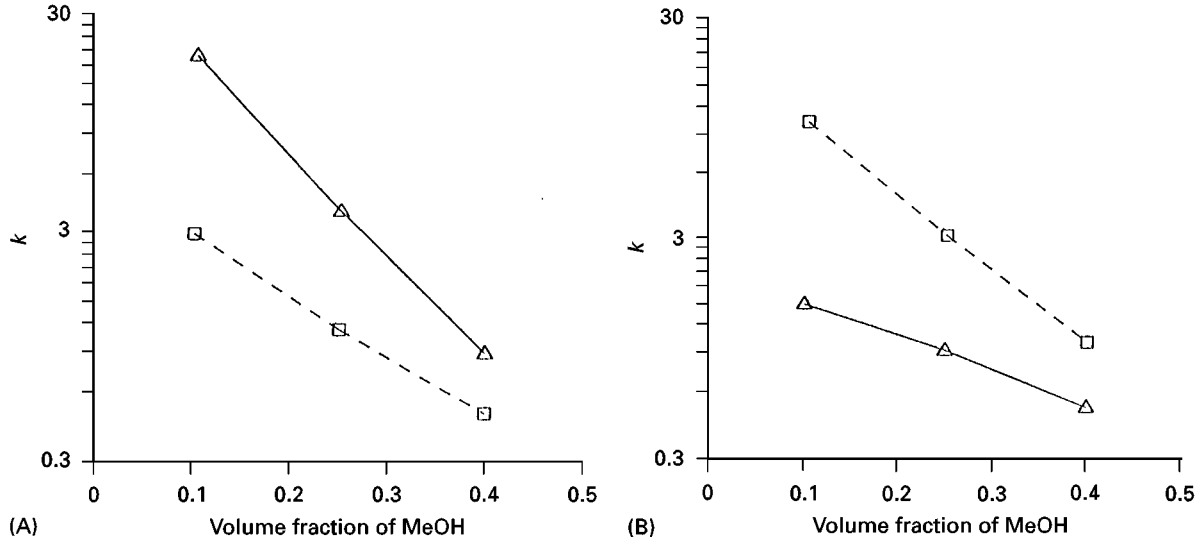

Figure 6 Retention factor for (A) phenylalanine and (B) naphthalene-2-sulfonic acid as a function of the methanol concentration (ϕ) in the phosphate buffer (pH 2.1 and constant ionic strength 175 mM Na^+) in the mobile phase in the absence (□) and presence (△) of 5 mM sodium octylsulfonate pairing ion. (Reproduced with permission from Bartha Á and Ståhlberg J (1994) *Journal of Chromatography A* 668: 255.)

sequently its retention. In a reversed-phase system, with no ion pair reagent added, the retention factor k_B is the weighted sum of the retention factor for the charged, k_{iB}, and the uncharged, k_{uB}, species, respectively:

$$k_B = (1 - f) \cdot k_{uB} + f \cdot k_{iB} \quad [8]$$

where f is the fraction of charged analyte in the mobile phase at the given pH value. Addition of an ion pair reagent to the mobile phase mainly influences the retention of the ionized fraction of the analyte. Furthermore, the influence can be treated with the same formalism as for a fully ionized analyte, i.e. eqn [8] takes the following form:

$$k_B = (1 - f) \cdot k_{uB} + f \cdot k_{0iB} \cdot \exp\left(-\frac{z_{iB} F \Psi_0}{RT}\right) \quad [9]$$

where k_{0iB} is the retention factor of the fully ionized form of the analyte in the absence of the ion pair reagent. **Figure 7** shows the retention of a weak base, adenine ($pK_a = 4.12$), as function of pH in the absence and in the presence of an oppositely charged ion pair reagent (5 mM sodium octylsulfonate).

Choice of Experimental Parameters in IPC

Large changes in retention of ionic analytes may occur when the pH, the concentration of organic modifier and the concentration of ion pair reagent is varied. The purpose of IPC is to take advantage of these variations in order to achieve a separation.

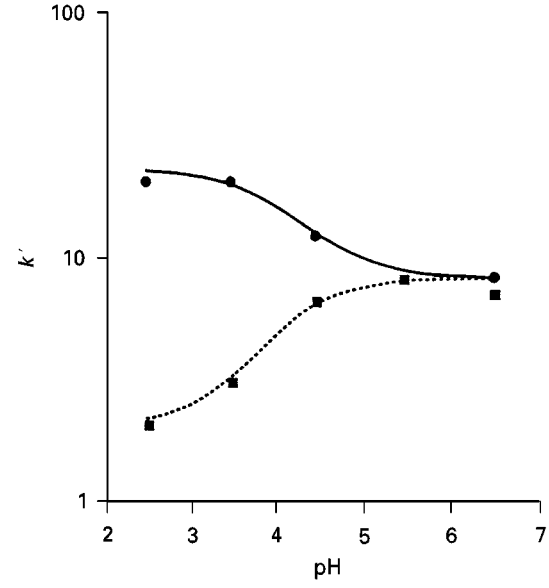

Figure 7 Retention factor for the weak base adenine ($z_{iB} = +1$) as a function of pH in the mobile phase in the absence (■) and presence (●) of the pairing ion sodium octylsulfonate. The theoretical retention plots in the absence of the pairing ion (dashed line, $\Psi_0 = 0$) and in its presence (solid line) are calculated from eqn [9] with the following parameters; $pK_a = 4.12$, $k_{uB} = 8.2$, $k_{iB} = 2.07$, $z_{iB} F \Psi_0 / RT = -2.398$. (Reproduced with permission from Bartha Á, Ståhlberg J and Szokoli F (1991) *Journal of Chromatography* 552: 13.)

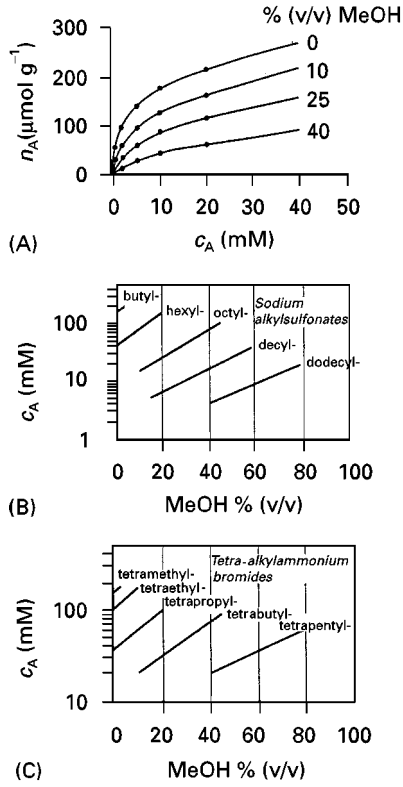

Figure 8 (A) Adsorption isotherm of octylsulfonate to a C_{18}-RP-column with the concentration of MeOH in the mobile phase as parameter. (B) Recommended alkylsulfonate type and concentration for different MeOH concentrations in the mobile phase, and (C) recommended tetraalkylammonium ion type and concentration for different MeOH concentrations in the mobile phase. (Reproduced with permission from Bartha Á, Vigh Gy and Varga-Puchony Z (1990) *Journal of Chromatography* 499: 423.)

This means that method development in this area usually is successful but requires a basic understanding of the physical processes that determine the retention.

In the development of an IPC method, the first point to consider is the relationship between the pK_a value of the analyte ions and the pH value of the mobile phase. To obtain a robust system the mobile phase pH should deviate from the pK_a value with more than two units. The pH is controlled by using a 10–50 mM salt buffer in the mobile phase. The buffer salt not only adjusts the pH but also controls the strength of electrostatic interaction between the ionic analytes and the ion pair reagent.

For changing the retention of an ionic analyte there are mainly three parameters to be varied: the type and mobile phase concentration of the ion pair reagent and the concentration of the organic modifier. The physically important parameter that is changed when these three parameters vary is the surface concentration of the ion pair reagent, and the corresponding electrostatic surface potential. A guideline for choosing type and concentration of positively and negatively charged ion pair reagents, respectively, as a function of the concentration of MeOH in the mobile phase is shown in **Figure 8**. The general rules for converting from MeOH to other common types of organic modifiers also apply in this case, e.g. 20% MeOH in the mobile phase corresponds to approximately 8% acetonitrile.

See also: **II/Chromatography: Liquid:** Mechanisms: Ion Chromatography; Mechanisms: Reversed Phases. **Chromatography: Thin-Layer (Planar):** Ion Pair Thin-Layer (Planar) Chromatography. **III/Chiral Separations:** Ion-Pair Chromatography. **Peptides and Proteins:** Liquid Chromatography.

Further Reading

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